THE TUBER-LIKE ROOTLETS OF CYCAS REVOLUTA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXVI.

A. C. LIFE.

[WITH TEN FIGURES]

THE coral-like outgrowths in connection with the roots of *Cycas revoluta* have long been known. In 1871 and 1872 Reinke[1] published an account of them and described the associated endophytic alga, which he referred to Anabaena (*fig. 10*). In 1894 Schneider[2] published further details, and referred to these structures as root tubercles. My own work was begun at Indiana University and continued at The University of Chicago.

The occurrence of these structures in *Cycas revoluta* is by no means uniform in greenhouse plants, as they are abundant in some cases, few in others, and entirely lacking in still others. They are connected with the upward rising rootlets, usually spreading radiately from the apex and just behind it, and are most abundant at or very near the surface of the ground, but may occur several inches beneath it. They vary considerably in size, but are always larger than the normal roots of the same age, and by branching may form clusters 2.5^{cm} or more in diameter (*fig. 1*).

This branching is apparently dichotomous and such a claim has been made for these rootlets. According to Reinke[3] this fact indicates relationship with the Lycopodiales, while Schneider[2] suggests that it is a case of atavism. If by true dichotomy it is

[1] Einige Bemerkungen über das Spitzenwachstum der Gymnospermen Wurzel. Gött. Nachrichten 530. 1871.

Parasitische Anabaena in Wurzeln der Cycadeen. Gött. Nachrichten 107. 1872.

[2] Mutualistic symbiosis of algae and bacteria with *Cycas revoluta*. BOT. GAZ. 19:25–32. *pls. 3, 4*. 1894.

[3] Beitrag zur Kenntniss der Gymnospermwurzel. Just's Bot. Jahresb. 1: 205–207. 1873.

meant that the whole of the apical meristem passes into the meristem of the branches, this is not a case of true dichotomy. At an early stage the meristems of the two branches become definitely outlined, but between them, retaining a central apical position, there remains a portion of the original meristem. This true apical meristem gives no indication of continuing the axis, and does not even begin to form bundle elements, but soon becomes indistinguishable, and the later stages of the rootlets show what seems to be a real dichotomy. The conclusion is that this is a case of apparent dichotomy in which the meristem of the main axis ceases to function almost as soon as the meristems of the two branches are differentiated from it. It may be of interest to note that some of the tubercled rootlets do not branch, and that the normal rootlets do not show even an apparent dichotomy.

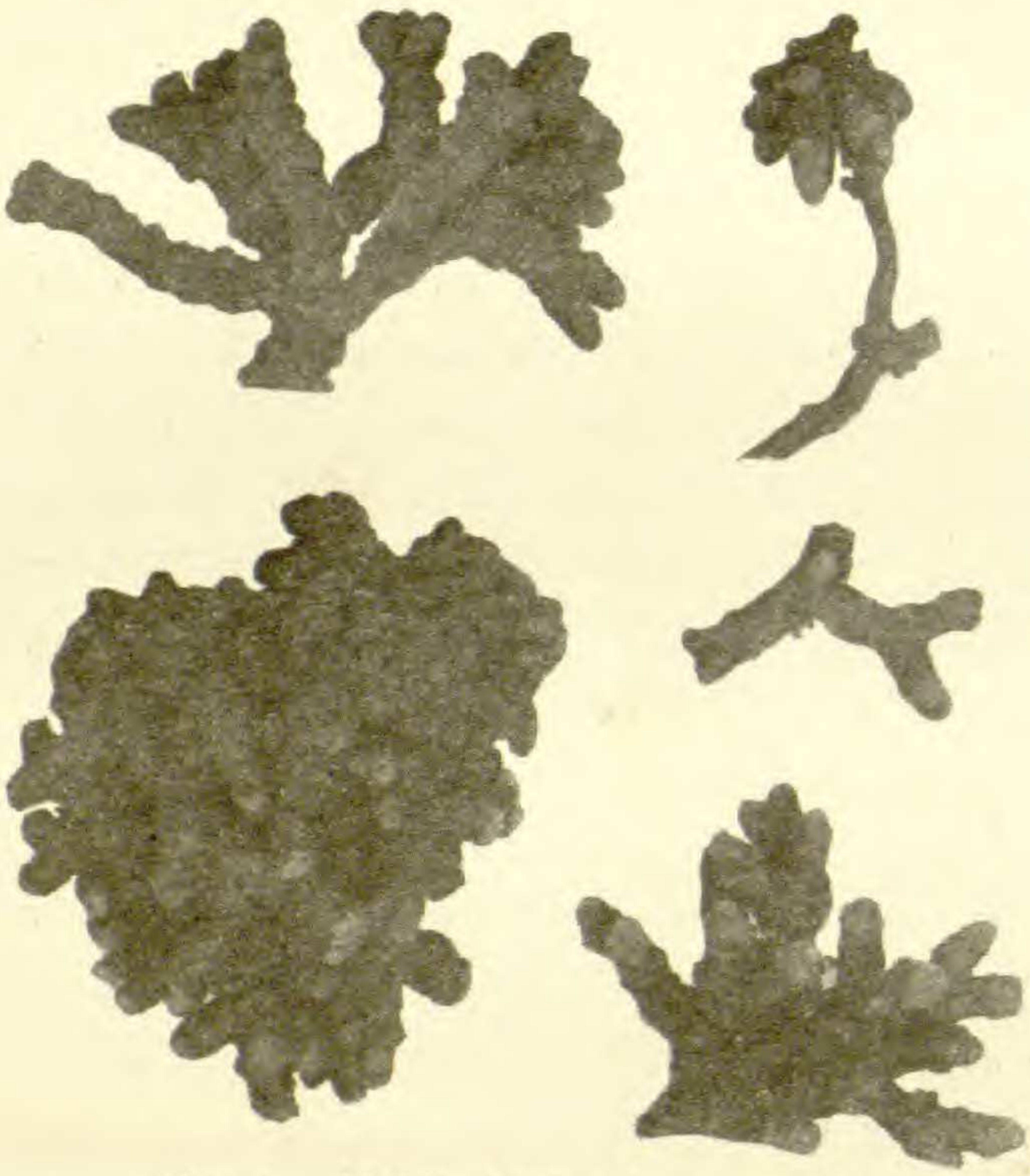


FIG. 1.— Habit of tubercles; nat. size.

A transverse section of one of the tubercles (*fig. 2*) shows a central vascular cylinder like that of the normal root, and a very thick cortex interrupted about midway by the greenish algal zone (*fig. 2, a*). The inner mass of cortex consists of ten or twelve layers of cells. The outer region is more differentiated, consisting of elongated parenchyma cells next to the algal zone, a zone of roundish cells with small intercellular spaces, a layer of cork cambium, and two layers of corky cells.

In longitudinal sections the algal zone is seen to extend from the base of the tubercle almost to the very apex (*fig. 3*). Where first distinguishable it consists of cells whose contents are somewhat denser than those of the neighboring cells, and before any intercellular spaces appear the cells next to the zone on both sides become very distinctly differentiated into a layer (*f*) resembling a tapetum. When fully developed the algal zone consists of loosely connected cells with large intercellular spaces which are occupied by the algae (*figs. 4, 5*). The radial elongation of these loosely connected cells induced Schneider to call them palisade tissue. Where large lenticels occur there is a break in the algal zone, and instead of the usual layers the round parenchyma cells extend from the phellogen to the vascular cylinder (*fig. 6*). The round cells with their small intercellular spaces form an effective air-conducting tissue.

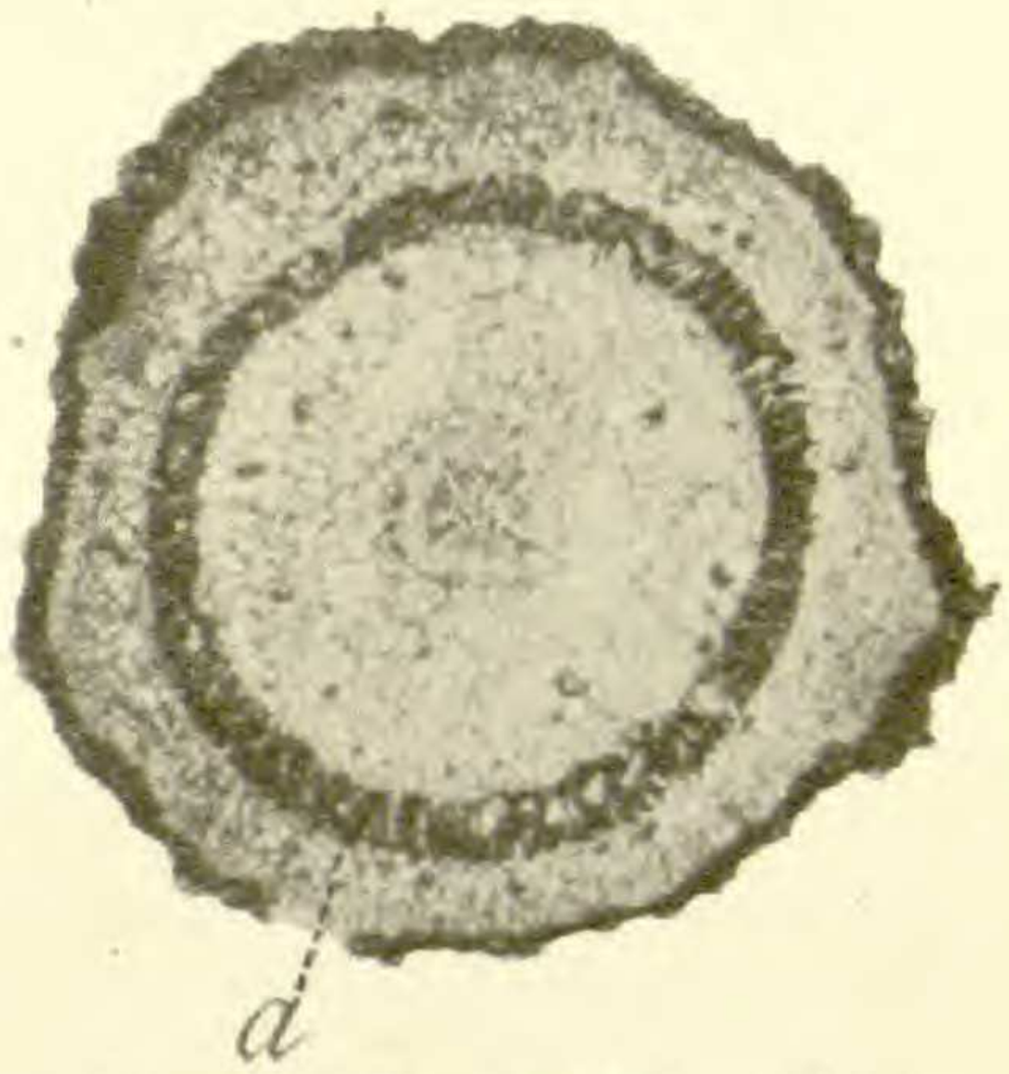


FIG. 2.—Transverse section of a tubercle. × 15. *a*, algal zone.

FIG. 3.—Longitudinal section of tubercle. × 40. *a*, algal zone; *f*, tapetum-like layer, with hyphae.

Lenticels occur in abundance upon the tubercles, and are found near the tip of a root upon which young tubercles are growing. They occur also in thickened primary roots which do not bear tubercles. That they are developed very early in the growth of a tubercle is evidenced by the fact that they may be seen forming very near the tip. To determine whether these structures are really lenticels, air was forced through the tubercles under water by means of an aspirator. The streams of air which were given off indicated that the structures are lenticels in function as well as in form. It might be inferred, therefore, that the so-called tubercles are used in aeration, but this would seem to be but an incidental result of their structure. It has been shown by Jost[4] that plants deprived of a sufficient supply of oxygen develop air conducting tissue and abnormal growths, and it is possible that this represents the condition of *Cycas*.

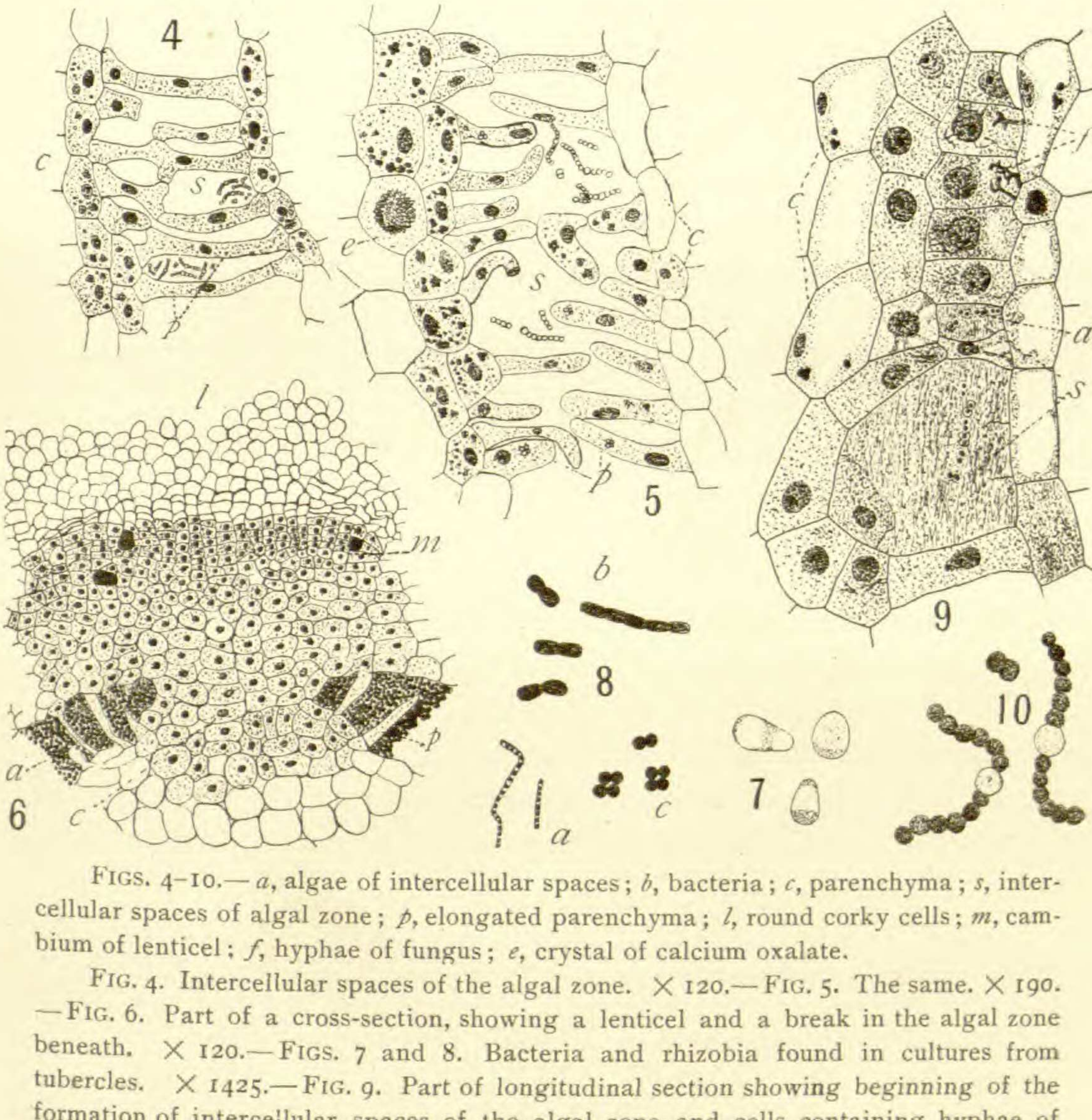
Upon comparing the general structure of the tubercle-like rootlets with those which are normal, it is to be noted that the tubercles have round tips which do not possess a true root cap. Instead of the conical root cap of the normal root they have a sheath of several cell layers extending over the tip and also enveloping the entire tubercle as an outer cortex (*fig. 3*). No such cortex is developed in the normal root.

In studying the fungi and bacteria of the tubercles, cultures were made on agar. From these cultures at least three bacterial forms and an organism resembling the Rhizobium of Schneider[2] were obtained (*figs. 7, 8*). The bacteria included one very large form, a small chain-like form, and a coccus form. The hyphae of fungi were also observed in the cells of the tubercles just in advance of the algal zone (*fig. 9*), but they were not in a condition to be identified. In fact, a zone of differentiated cells extends from the algal zone to the meristem of the tip (*fig. 3*), the cells being shorter than the adjoining cells and their contents more granular.

The fungi and bacteria which are in the cells in advance of the algal zone seem to prepare the way for the algae (*fig. 10*), since their presence seems to result in very much enlarging the small intercellular spaces, which become the relatively large

[4] Ein Beitrag zur Kenntniss der Athmungsorgane der Pflanzen. Bot. Zeit. **45**: 37–39. 1887.

chambers occupied by the algae. The presence of the fungi and bacteria within the cells seems to retard the nutritive work, so that the cells cannot keep pace with those adjacent. This produces tension which results in a development of spaces, and by



FIGS. 4–10.— *a*, algae of intercellular spaces; *b*, bacteria; *c*, parenchyma; *s*, intercellular spaces of algal zone; *p*, elongated parenchyma; *l*, round corky cells; *m*, cambium of lenticel; *f*, hyphae of fungus; *e*, crystal of calcium oxalate.

FIG. 4. Intercellular spaces of the algal zone. × 120.— FIG. 5. The same. × 190. — FIG. 6. Part of a cross-section, showing a lenticel and a break in the algal zone beneath. × 120.— FIGS. 7 and 8. Bacteria and rhizobia found in cultures from tubercles. × 1425.— FIG. 9. Part of longitudinal section showing beginning of the formation of intercellular spaces of the algal zone, and cells containing hyphae of fungus. × 300.— FIG. 10. Algae found in the tubercle. × 480.

means of this tension some of the cells are broken, while others appear to be broken down by the organisms within them. After the algae have gained entrance, their growth and multiplication probably result in still further increasing the size of these intercellular spaces, since they become larger the further they occur from the region of origin.

In the early stages of the growth of the tubercles, before they contain the algae, numerous lenticular areas may be observed at their bases and also upon the adjacent part of the root which bears them. These peculiar areas have openings in them which appear as crevices, and as the tubercles grow older the whole area frequently breaks away, and it is doubtless through these rents that the algae effect an entrance. Together with the algae the fungi and bacteria also enter, the latter forms penetrating the cells and causing the enlargement of the intercellular spaces.

In reference to the symbiotic relations which exist between these various organisms it is difficult to speak with any certainty. The fungi and bacteria doubtless find congenial conditions of moisture and food in connection with the algae, and are in turn the principal agents in producing the intercellular spaces in which the algae thrive. It is barely possible that there may be some such chemical attraction between the fungi and algae and the cells of the differentiated layer as has been stated by Miyoshi.[5] It has been demonstrated by Vines,[6] Frank,[7] MacDougal,[8] and others that fungi growing upon the surfaces of cells or within them may aid in nutritive work by converting free nitrogen and the simpler nitrogen compounds into the more complex forms used by the plant. The same function has been attributed to certain algae by Prantl,[9] especially including the nostoc forms. This last observation suggests the possibility of the use

[5] Ueber Chemotropismus der Pilze. Bot. Zeit. **52** : 1–28. *pl. 1*. 1894.

[6] On the relation of the formation of tubercles on the roots of Leguminosae and the presence of nitrogen in the soil. Ann. Bot. **2** : 386–389. 1888.

[7] Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. deut. bot. Gesell. **3** : 128–145. *pl. 10*. 1885.

[8] Symbiotic saprophytism. Ann. Bot. **13** : 1–47. *pls. 1, 2*. 1899.

[9] Die Assimilation freien Stickstoffes und der Parasitismus von Nostoc. Hedwigia **28** : 135. 1889.

of the nostoc forms within the tubercles of the cycads in assisting nitrogen assimilation.

In conclusion, therefore, the tubercles of cycads may be said to have at least two functions, that of aerating, and that of assisting in nitrogen assimilation.

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